Evaluating the effectiveness of sodium hypochlorite for genomic DNA decontamination

Environmental DNA

WILEY

Environmental DNA
Open Access
Dedicated to the study and use of environmental DNA for basic and applied sciences

ORIGINAL ARTICLE OPEN ACCESS

Evaluating the Effectiveness of Sodium Hypochlorite for Genomic DNA Decontamination

Ashinsa de Silva Wijeyeratne | Hyun S. Gweon

School of Biological Sciences, University of Reading, Reading, UK

Correspondence: Hyun S. Gweon (h.s.gweon@reading.ac.uk)

Received: 8 July 2024 | **Revised:** 19 November 2024 | **Accepted:** 6 January 2025

Funding: The authors received no specific funding for this work.

ABSTRACT

Environmental DNA (eDNA) is an increasingly popular, sensitive, and cost-efficient method for studying biodiversity and detecting species. This noninvasive approach involves collecting environmental samples that contain genetic material shed by organisms into their surroundings. Due to the method's sensitivity, robust decontamination strategies are crucial, with sodium hypochlorite, commonly known as bleach, frequently employed. Despite its widespread use, there is no consensus on the most effective bleach concentration, leading to inconsistencies in how the chemical is used in research. This study aimed to determine the minimum concentration of bleach needed for effective decontamination. Genomic DNA of signal crayfish was treated with various concentrations of bleach, ranging from 0.01% to 5% (w/w). Results were observed using Qubit High Sensitivity reagents, quantitative PCR, agarose gel electrophoresis, and the Agilent TapeStation. Our results indicate that a minimum concentration of 0.5% (w/w) bleach is sufficient to prevent the detection of genomic DNA by the techniques tested. These results provide important insights into the use of bleach for decontamination in eDNA research. Establishing a standard bleach concentration for decontamination protocols will help to reduce inconsistencies and enhance the reliability of eDNA studies.

1 | Introduction

DNA has emerged as an invaluable tool with diverse applications spanning healthcare, agriculture, forensics, and data storage. Notably, in the past decade, the utilization of environmental DNA (eDNA) has gained popularity for conducting biodiversity assessments and species monitoring (Bohmann et al. 2014; Thomsen and Willerslev 2015; Miya 2022). When integrated with conventional survey methods, eDNA plays an instrumental role in the identification and monitoring endangered species (Mauvisseau et al. 2020), invasive species (Piaggio et al. 2014) and the inference of the presence of "dark taxa"—or taxa that are seldom observed and remain poorly understood (Boussarie et al. 2018; Saccò et al. 2022). Noteworthy advantages of eDNA include nondestructive sampling and minimal stress on the sampled organisms, allowing for the detection of species with low population densities (Díaz-Ferguson and Moyer 2014). Given the delicate nature of eDNA studies, the implementation of reliable decontamination strategies is crucial (Goldberg et al. 2016).

DNA pervades natural environments ubiquitously, necessitating robust decontamination processes to eliminate unwanted DNA and prevent confounding, ambiguous or erroneous outcomes. Researchers across various disciplines have implemented diverse techniques and best practices to mitigate DNA contamination. Common methods include UV exposure (Gefrides et al. 2010), 70% ethanol treatment (Hoffmann, Fingerle, and Noll 2020) and sodium hypochlorite, commonly known as bleach (Champlot et al. 2010; Huszarik et al. 2023). Several studies have compared the effectiveness of various reagents and products, identifying bleach-based products as the most effective decontamination agents, albeit with variations in application concentration, across diverse context such as surface decontamination of materials ranging from wood to insect gut

contents, and within forensic settings (Prince and Andrus 1992; Greenstone et al. 2012; Fischer et al. 2016; Nilsson, de Maeyer, and Allen 2022; Stoufer et al. 2023).

In eDNA studies, the potency of bleach used varies widely, ranging from 1% to 20%. For example, a study monitoring microbial and arthropod communities associated with the Western honeybee used 1% bleach to decontaminate all targeted sampling surfaces of preexisting eDNA prior to collecting fresh eDNA (Boardman et al. 2024). Another study comparing effective capture and storage methods for aqueous macrobial eDNA decontaminated the buckets used for water collection by soaking them in 5% bleach (Spens et al. 2017). Additionally, filtration and collection equipment were decontaminated with 20% bleach prior to sampling water of both high and low turbidity (Kumar et al. 2022). Variation of bleach concentration was also reported within the same study, where 2% household bleach was used to decontaminate an inflatable kayak, life jacket and paddle, whereas water filtering manifolds and similar equipment were decontaminated with 30% household bleach (Yates et al. 2021). A review of 75 published eDNA studies found that 59 did not specify any details regarding decontamination of sampling equipment, and only two specified the use of bleach yet without detailing the concentration (Dickie et al. 2018). To date, there is no common consensus regarding the usage of bleach in eDNA studies, especially regarding the most effective minimum concentration for decontamination, and a comprehensive exploration into the most effective minimum concentration of sodium hypochlorite for eliminating DNA in an eDNA context is still pending.

In this study, we conducted an investigation aimed at addressing the aforementioned gap in existing literature regarding the impact of bleach on DNA decontamination within the field of eDNA. We focused on determining the minimum concentration of sodium hypochlorite required to completely inhibit the detection of genomic DNA utilizing common molecular methodologies and reagents prevalent in the eDNA field. Additionally, we aimed to observe the extent of double-stranded DNA fragmentation following exposure to bleach. The overarching objective of this study is to provide practitioners and researchers engaged in eDNA analysis with critical insights, thereby enabling them to make informed decisions regarding the implementation of appropriate decontamination strategies in their experimental protocols.

2 | Materials and Methods

2.1 | Preparation of DNA and Sodium Hypochlorite

Genomic DNA was extracted from the leg tissues of a frozen specimen of signal crayfish (*Pacifastacus leniusculus*) using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The eluted DNA was quantified using the Qubit 3.0 Fluorometer (Invitrogen). A commercially available, laboratory-grade sodium hypochlorite (bleach) (CHE3456, Scientific Laboratory Supplies Ltd) was diluted with appropriate volumes of molecular-grade water (UltraPure, Invitrogen) to prepare bleach solutions with available chlorine concentrations of 0.02%, 0.1%, 0.2%, 1.0%, 2%, and 10% (w/w). Subsequently, 20 μL of the extracted genomic DNA at 50 ng/μL was exposed to bleach by adding 20 μL of each bleach concentration to achieve final bleach concentrations of 0.01%, 0.05%, 0.1%, 0.5%, 1%, and 5%. Additionally, 20 μL of untreated genomic DNA was included as a no treatment control. The mixtures were then incubated at room temperature for 30 min, with all samples prepared in triplicate.

2.2 | Ethanol Precipitation of DNA

In order to remove sodium hypochlorite for the subsequent amplification step, a modified version of the standard DNA ethanol precipitation protocol was used. Specifically, 20 μL aliquots from each of the above mixtures were combined with 380 μL of molecular-grade water. 40 μL of 3 M sodium acetate (Thermo Fisher Scientific) and 800 μL of ice-cold absolute ethanol (Fisher Scientific) were added to each sample. 1200 μL of the same absolute ethanol was added to 40 μL of sodium acetate as a negative control. Following the addition of reagents, all samples were incubated at −20°C for 30 min, followed by centrifugation for 60 min at 4°C and 12,000 *g*. Subsequently, 1220 μL of the supernatant was carefully removed from the top and quantified using the Qubit High Sensitivity reagents on the Qubit fluorometer (Invitrogen). The remaining 20 μL of each sample was subjected to vacuum drying at 45°C using a Centrivap Concentrator (Labconco). Post-drying, samples were resuspended in 20 μL of molecular-grade water, vortexed thoroughly, and quantified using Qubit High Sensitivity reagents.

2.3 | Agarose Gel Electrophoresis

A 1.5% agarose gel was prepared using molecular grade agar (Meridian Bioscience) and electrophoresed in a buffer of sodium borate at 240 V for 20 min. Subsequently, the get was visualized and captured using the Syngene T:Genius imaging system in conjunction with GeneSys Software V1.5.5.0 (Syngene).

2.4 | TapeStation Analysis

The fragmentation analysis of genomic DNA was conducted using the Agilent 4200 TapeStation (Agilent Technologies), an automated electrophoresis platform that assesses DNA quality and quantity. Specifically, the Genomic DNA Screen Tape, designed to detect genomic DNA ranging from 200 to 60,000 base pairs (bp), was used. Peaks observed in the output graph corresponded to the size (in bp) and quantity (measured as fluorescent units or FU) of DNA fragments within the sample.

Sample preparation and analysis procedures followed the manufacturer's instructions. Briefly, for each 1 μL of samples and Genomic DNA ladder, 10 μL of sample buffer (Agilent Technologies) was mixed and loaded into the machine. A new Genomic DNA Screentape (Agilent Technologies) was loaded into the machine, along with the necessary tips (Agilent Technologies). Each experimental concentration of bleach was represented by one sample, in addition to samples for no treatment and negative controls. Supplementary samples included

molecular-grade water and genomic DNA that had not undergone ethanol purification. The obtained results were analyzed and visualized using the TapeStation Analysis Software (Agilent Technologies, v.4.1.1) and bioanalyzeR (https://github.com/jwfoley/bioanalyzeR, v.0.10.0), respectively.

2.5 | Quantitative PCR (qPCR)

Quantitative PCR (qPCR) was carried out for the detection of signal crayfish genomic DNA. Primer pairs targeting a 114 bp region of cytochrome C oxidase subunit 1 (CO1) mitochondrial gene of signal crayfish, namely CO1-Pl-02-F (TGAGCTGGTATAGTGGGAACT) and CO1-Pl-02-R (AGCATGTGCCGTGACTACAA) (Mauvisseau et al. 2018) were used for amplification. The assays were performed on the StepOnePlus Real time PCR System (Applied Biosystems) under the following thermal cycling conditions: initial pre-holding at 56°C for 30 s, holding at 50°C for 5 min, denaturation at 95°C for 10 min, followed by 55 cycles of denaturation at 95°C for 30 s and extension at 56°C for 1 min. Post-PCR holding was at 56°C for 30 s. Each sample was subjected to three PCR replicates, with each qPCR reaction containing 12.5 μL volume, comprising 6.25 μL of Environmental Mastermix (Applied Biosystems), 1.25 μL of molecular-grade water, 0.5 μL (10 μM) of each primer, 0.5 μL (2.5 μM) of TaqMan probe (Merck), 1.25 μL of 10× Exo IPC Mix (Thermo Fischer Scientific), 0.25 μL of 50× Exo IPC DNA mix (Thermo Fisher Scientific) and 2 μL of experimental sample. Additionally, six no-template controls (NTCs) were included, using 2 μL of nuclease-free distilled water instead of the experimental sample. Three no amplification controls (NACs) were included, where 2 μL of 10× IPC Blocker (Thermo Fischer Scientific) replaced the experimental sample. For comparative analysis, a series of triplicate serial dilutions ranging from 10^{-1} to 10^{-6} of signal crayfish genomic DNA was also run in parallel with the experimental samples. The cycle threshold (Ct) values of each qPCR sample were recorded, and presence/absence was determined based on these results.

3 | Results

3.1 | Fluorometric Quantification (Qubit) of Bleach-Treated gDNA

The mean DNA concentration observed in samples treated with 0.01% bleach was the highest of the treated groups, measuring at 8.40 ng/μL (Figure 1, Table 1). DNA treated with 0.05% bleach was the second highest at 8.09 ng/μL, followed by 0.1% treatment at 3.80 ng/μL. The untreated DNA had a mean concentration of 10.96 ng/μL. A decreasing trend in DNA concentration was observed across the treatments. DNA was detected in all replicates subjected to bleach concentrations 0.01%, 0.05%, 0.1%, and in untreated DNA. All samples treated with 0.5% bleach or higher did not yield quantifiable DNA concentrations.

3.2 | Visualization of Bleach-Treated gDNA on Agarose Gel Electrophoresis

Bands were visible in the samples treated with 0%, 0.01%, 0.05%, and 0.1% bleach whereas no bands were present in those treated with bleach at concentrations of 0.5% and higher (Figure S1).

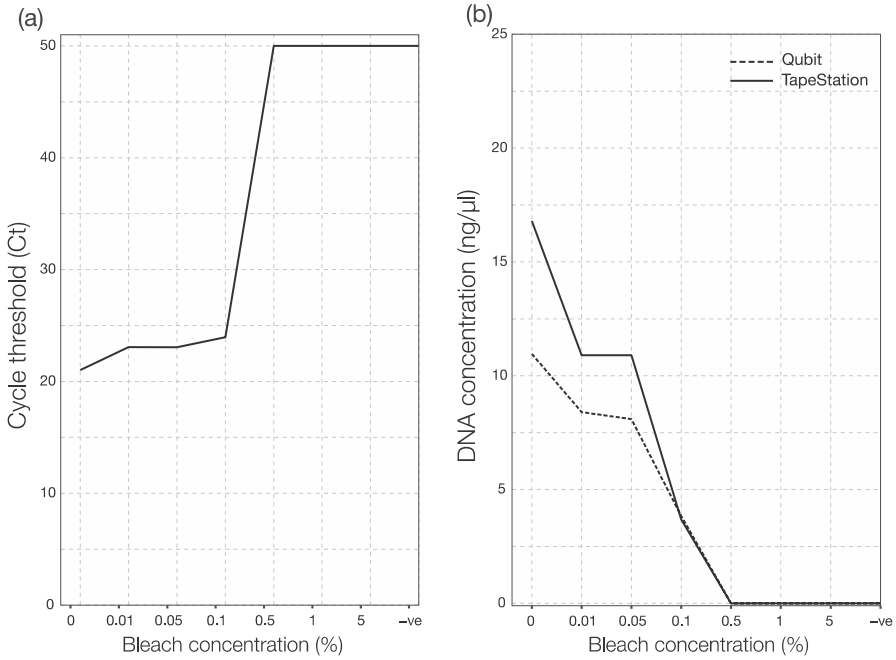

FIGURE 1 | Detection of genomic DNA of signal crayfish across a range of bleach treatment concentrations from 0% to 5%, along with a negative control using (a) quantitative PCR (qPCR); and (b) Quantification of DNA using the Qubit fluorometer (dotted line), and the Agilent 4200 TapeStation (solid line). Across all methods, DNA was consistently detected in samples treated with 0%, 0.01%, 0.05%, and 0.1% bleach, whereas DNA was undetected in 0.5% and higher. Ct values increased (indicating less detectable DNA), and DNA concentration decreased with increasing bleach treatment concentration.

TABLE 1 | Quantification of double-stranded DNA concentration using the Qubit fluorometer (High Sensitivity) across various bleach treatment concentrations, ranging from 0% (untreated) to 5%, along with a negative control.

Sample	DNA concentration (ng/μL)
Untreated DNA (0% bleach)	10.96
DNA treated with 0.01% bleach	8.40
DNA treated with 0.05% bleach	8.09
DNA treated with 0.1% bleach	3.80
DNA treated with 0.5% bleach	Too low to be measured
DNA treated with 1% bleach	Too low to be measured
DNA treated with 5% bleach	Too low to be measured
Negative control	Too low to be measured

Note: Quantifiable DNA was observed in samples treated with 0%, 0.01%, 0.05%, and 0.1% bleach, showing a gradual decline in concentration as bleach concentration increased. Samples treated with 0.5% bleach or higher showed no detectable DNA. Concentrations are expressed in ng/μL.

3.3 | Assessment of Bleach-Treated gDNA on the TapeStation

The TapeStation analysis detected the presence of DNA in samples treated with 0.01%, 0.05% and 0.1% bleach, with fragment sizes peaking at 5387 bp, 5722 bp, and 5153 bp, respectively. Samples of 0.5%, 1%, and 5% did not show any peaks within the detectable range of the instrument, nor did the negative controls of ethanol and water (Figure 2). Readings for DNA concentrations are shown in Table 2 and Figure 1B. There was an overall trend of decreasing DNA concentration as the bleach concentration increased. The untreated sample of DNA, that is, untreated but purified with ethanol precipitation, had a concentration of 16.80 ng/μL, whereas DNA not subjected to ethanol purification showed 52.80 ng/μL.

3.4 | Presence or Absence Determination Using qPCR

In all nine replicates of bleach concentrations at 0.01%, 0.05%, and 0.1%, signal crayfish DNA was consistently detected using qPCR. The average Ct values for positive samples ranged from 23.07 to 23.95. A detection threshold was set at an average Ct value of 50 (Figure 1A, Table 3). The Internal Positive Control (IPC) was successfully amplified in all samples, and no amplification signal was observed in the No Template Controls (NTCs). Additionally, the IPC was effectively blocked in all No Amplification Controls (NACs), affirming the reliability of the experimental procedures in ensuring accurate detection and preventing false positives. In the dilution series, DNA remained detectable until 10^{-4}, with Ct values gradually increasing to 23.43, 26.99, 32.98, and 40.16. However, no DNA was detected in any replicate at dilutions of 10^{-5} and 10^{-6}.

4 | Discussion

The field of eDNA, often reliant on detecting trace amounts of DNA from environmental samples, necessitates robust decontamination protocols to ensure accurate and reliable results. Although practitioners commonly use bleach for decontamination, there is no consensus on the most appropriate bleach concentration for eDNA protocols. High concentrations of bleach, which is corrosive and an irritant, risk damaging equipment and clothing, and pose health and safety concerns, whereas low concentrations may lead to inaccurate results. In this study, we investigated the impact of various bleach concentrations from 0.01% to 5% on genomic DNA, utilizing the Qubit Flourometer, Agilent 4200 TapeStation, gel electrophoresis, and qPCR. Consistent results across all methodologies demonstrated that bleach concentrations of 0.5% and higher effectively inhibited DNA detection and amplification.

Notably, fragment size analysis using the Agilent 4200 TapeStation showed peaks at 5000 bp for samples treated with up to 0.1% bleach, but none for concentrations of 0.5% and above. This absence of peaks was unexpected, as bleach was anticipated to fragment DNA into smaller pieces. Although the minimum detection level of double-stranded genomic DNA for the Agilent 4200 Tapestation is 200 bp, suggesting the possibility that bleach fragmented DNA into pieces smaller than 200 bp, the complete absence of the target locus in the qPCR assay, as well as no detectable levels of double-stranded DNA from the Qubit Fluorometer and lack of visible bands on the agarose gel, possibly suggest that DNA is modified into a undetectable form rather than being fragmented.

Despite its well-established sterilization capabilities, the precise mechanism by which bleach "eliminates" DNA remains elusive. Hypochlorite, the active chemical in bleach, is a potent oxidizing agent known for its sterilization effects achieved through irreversible alterations to proteins (Winter et al. 2008). There are several possible mechanisms for DNA degradation upon exposure to bleach. The chlorination of nitrogenous bases, combined with the free radicals generated through oxidation, leads to the destruction of these bases, rendering them undetectable (Whiteman, Jenner, and Halliwell 1997; Osinnikova, Moroshkina, and Mokronosova 2019). It has also previously been suggested that bleach induces strand breakage in DNA (Ohnishi, Murata, and Kawanishi 2002). Additionally, treatment with bleach has been observed to enhance the propensity for cytosine deamination, a hydrolysis reaction wherein cytosine is substituted by thymine (Riley 2019). Chromatographic

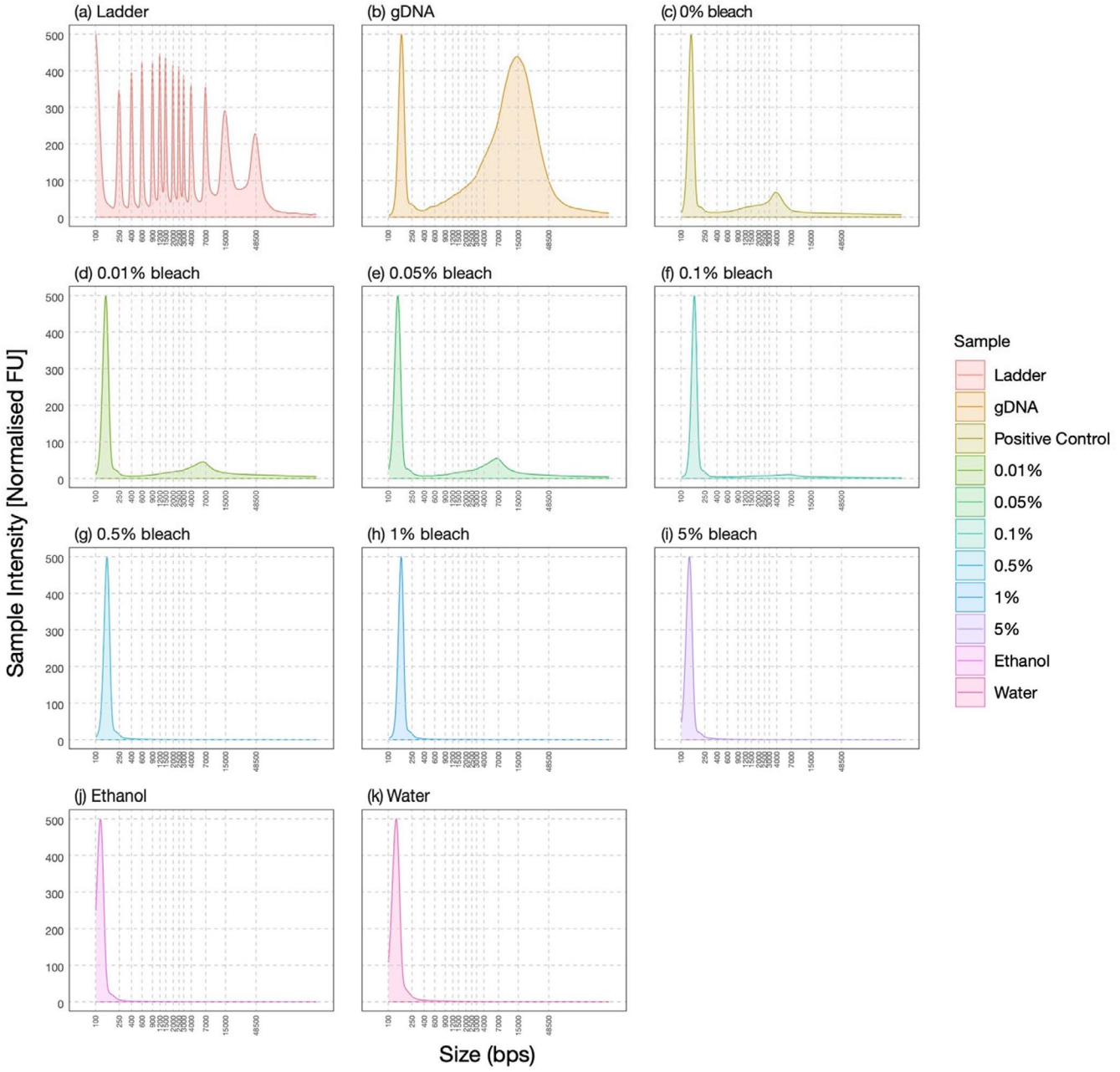

FIGURE 2 | Size distributions analyzed with the Agilent 4200 TapeStation. (a) The TapeStation ladder; (b) raw genomic DNA; (c–i) genomic DNA treated with bleach at various concentrations from 0% to 5%; (j, k) ethanol and water used during purification. The peak at 100 bps represents an internal standard. The y-axis measures sample intensity against DNA fragment sizes in base pairs (bp), that is, peaks indicate the amount of DNA at different sizes. Samples treated with 0.5%, 1%, and 5% bleach, as well as ethanol and water controls, did not show any detectable peaks.

analysis of nucleic acids treated with hypochlorite has demonstrated rapid and nonspecific reactions, resulting in the production of complex base derivatives, including 5-chlorouracil and 5-chlorocytosine (Hayatsu, Pan, and Ukita 1971).

Various DNA quantification and detection methods were employed in this study, all revealing a consistent decline in detected DNA concentration with increasing bleach concentration. However, differences were observed, reflecting the variability inherent in DNA quantification methods and techniques (Eichmiller, Miller, and Sorensen 2016; Spens et al. 2017; Peixoto et al. 2021). Comparative studies, such as the UK Biobank Genotyping project, indicate varied results among quantification methods (Welsh et al. 2017). Ancient DNA quantification comparisons showed the NanoDrop UV spectrophotometer and Qubit fluorometer reported higher concentrations than qPCR (Brzobohatá et al. 2017). Discrepancies were also observed in the analysis of degraded DNA, spanning fluorescent dye-based techniques, spectrophotometry, and qPCR (Shokere, Holden, and Ronald Jenkins 2009). When detecting striped bass fish eDNA, a comparison was made between two qPCR platforms: a handheld Biomeme three9 and a conventional benchtop Mic thermal cycler, where the latter produced higher concentration estimates and fewer false negatives (Skinner et al. 2020). These findings underscore the complexity of DNA quantification and the need for methodological consistency in eDNA studies.

TABLE 2 | Quantification of genomic DNA concentration recorded using the Agilent 4200 TapeStation across a range of treatment concentrations of bleach from 0% to 5%, alongside a negative control of ethanol.

Sample	DNA concentration (ng/μL)
Untreated DNA (0% bleach)	16.80
DNA treated with 0.01% bleach	10.90
DNA treated with 0.05% bleach	10.90
DNA treated with 0.1% bleach	3.68
DNA treated with 0.5% bleach	N/A
DNA treated with 1% bleach	N/A
DNA treated with 5% bleach	N/A
Negative control	N/A
Unpurified genomic DNA	52.80

Note: Pure water and unpurified genomic DNA that was not subjected to ethanol precipitation were included as additional controls. Quantifiable DNA was observed in the samples treated with up to 0.1% bleach as well as in the unpurified genomic DNA. No detectable DNA was seen in the water, ethanol and samples treated with 0.5% or higher bleach concentration.

TABLE 3 | Quantitative PCR (qPCR) results for the exposure of signal crayfish genomic DNA to bleach concentrations ranging from 0% to 5%.

Sample	Presence	Ct value
Untreated DNA (0% bleach)	Y	21.027
DNA treated with 0.01% bleach	Y	23.072
DNA treated with 0.05% bleach	Y	23.065
DNA treated with 0.1% bleach	Y	23.953
DNA treated with 0.5% bleach	N	N/A
DNA treated with 1% bleach	N	N/A
DNA treated with 5% bleach	N	N/A
Negative control	N	N/A

Note: DNA was detected up to 0.1% treatment, but not in higher treatment concentrations or the negative control. Across the board, Ct value showed a gradual increase alongside increasing treatment concentration.

Several limitations of the study exist. Firstly, varying concentrations of genomic DNA were not tested, nor was a variation in exposure time explored. Additionally, the fixed volume of bleach tested at 20 μL does not represent the variability that may occur in real-world field or laboratory settings during equipment decontamination. Furthermore, only dissolved DNA was tested, and not DNA bound to surfaces or materials such as filters or tubing, which may require different treatment protocols due to the properties of these materials. Moreover, further investigation is needed to determine the most effective concentration of bleach for eliminating PCR products or a higher load of DNA, especially for PCR amplicons or smaller DNA fragments with a high copy number.

Our findings demonstrate that a concentration of 0.5% (w/w) bleach at a very low volume effectively renders genomic DNA at a concentration of 50 ng/μL undetectable, even with highly sensitive methods such as qPCR. Based on these results, we recommend the use of bleach concentrations of 0.5% or preferably higher (e.g., 1%) for a minimum duration of 30 min to eliminate DNA contamination in both field and laboratory-based eDNA studies, regardless of whether DNA is fragmented or modified. Additionally, caution is advised when using household bleach for decontaminating equipment, as labeling practices differ between manufacturers. Some labels specify the percentage of available chlorine, whereas others indicate the sodium hypochlorite content. The conversion between sodium hypochlorite concentration and available chlorine may vary slightly, depending on the specific density of the sodium hypochlorite solution. Most household bleach solutions contain approximately 5% sodium hypochlorite, generally equivalent to 6%–7% available chlorine, and available chlorine in bleach degrades over time (Gow et al. 2022). Therefore, appropriate dilution is necessary, depending on the type of household bleach used. Lastly, due to the reactive and unstable nature of the hypochlorite ion, a significant portion of residual bleach after decontamination is likely to decompose or react with other substances in the environment. However, it remains prudent to ensure that all bleach is thoroughly rinsed off with water that has been verified to be free of the target species.

Author Contributions

Ashinsa de Silva Wijeyeratne acquired, analyzed and interpreted the experiments and data; Hyun S. Gweon conceived and designed the study; Ashinsa de Silva Wijeyeratne and Hyun S. Gweon wrote and edited the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

References

Boardman, L., J. A. P. Marcelino, R. E. Valentin, H. Boncristiani, J. M. Standley, and J. D. Ellis. 2024. "Novel eDNA Approaches to Monitor Western Honey Bee (*Apis mellifera* L.) Microbial and Arthropod Communities." *Environmental DNA* 6, no. 1: 419. https://doi.org/10.1002/edn3.419.

Bohmann, K., A. Evans, M. T. P. Gilbert, et al. 2014. "Environmental DNA for Wildlife Biology and Biodiversity Monitoring." *Trends in Ecology & Evolution* 29, no. 6: 358–367. https://doi.org/10.1016/J.TREE.2014.04.003.

Boussarie, G., J. Bakker, O. S. Wangensteen, et al. 2018. "Environmental DNA Illuminates the Dark Diversity of Sharks." *Science Advances* 4, no. 5: 9661. https://doi.org/10.1126/sciadv.aap9661.

Brzobohatá, K., E. Drozdová, J. Smutný, T. Zeman, and R. Beňuš. 2017. "Comparison of Suitability of the Most Common Ancient DNA Quantification Methods." *Genetic Testing and Molecular Biomarkers* 21, no. 4: 265–271. https://doi.org/10.1089/gtmb.2016.0197.

Champlot, S., C. Berthelot, M. Pruvost, E. Andrew Bennett, T. Grange, and E. M. Geigl. 2010. "An Efficient Multistrategy DNA

Decontamination Procedure of PCR Reagents for Hypersensitive PCR Applications." *PLoS One* 5, no. 9: e13042. https://doi.org/10.1371/journal.pone.0013042.

Díaz-Ferguson, E. E., and G. R. Moyer. 2014. "History, Applications, Methodological Issues and Perspectives for the Use of Environmental DNA (eDNA) in Marine and Freshwater Environments." *Revista de Biología Tropical* 62, no. 4: 1273–1284. https://doi.org/10.15517/rbt.v62i4.13231.

Dickie, I. A., S. Boyer, H. L. Buckley, et al. 2018. "Towards Robust and Repeatable Sampling Methods in eDNA-Based Studies." *Molecular Ecology Resources* 18, no. 5: 940–952. https://doi.org/10.1111/1755-0998.12907.

Eichmiller, J. J., L. M. Miller, and P. W. Sorensen. 2016. "Optimizing Techniques to Capture and Extract Environmental DNA for Detection and Quantification of Fish." *Molecular Ecology Resources* 16, no. 1: 56–68. https://doi.org/10.1111/1755-0998.12421.

Fischer, M., N. Renevey, B. Thür, D. Hoffmann, M. Beer, and B. Hoffmann. 2016. "Efficacy Assessment of Nucleic Acid Decontamination Reagents Used in Molecular Diagnostic Laboratories." *PLoS One* 11, no. 7: e0159274. https://doi.org/10.1371/journal.pone.0159274.

Gefrides, L. A., M. C. Powell, M. A. Donley, and R. Kahn. 2010. "UV Irradiation and Autoclave Treatment for Elimination of Contaminating DNA From Laboratory Consumables." *Forensic Science International. Genetics* 4, no. 2: 89–94. https://doi.org/10.1016/j.fsigen.2009.06.008.

Goldberg, C. S., C. R. Turner, K. Deiner, et al. 2016. "Critical Considerations for the Application of Environmental DNA Methods to Detect Aquatic Species." *Methods in Ecology and Evolution* 7, no. 11: 1299–1307. https://doi.org/10.1111/2041-210X.12595.

Gow, C., C. Weinhouse, G. Johnson, and K. Saunders. 2022. "Stability of Free Available Chlorine Levels in Dilute Sodium Hypochlorite Solutions Over a 6-Week Period." *Journal of the American Association for Laboratory Animal Science* 61, no. 2: 181–187. https://doi.org/10.30802/AALAS-JAALAS-21-000080.

Greenstone, M. H., D. C. Weber, T. A. Coudron, M. E. Payton, and J. S. Hu. 2012. "Removing External DNA Contamination From Arthropod Predators Destined for Molecular Gut-Content Analysis." *Molecular Ecology Resources* 12, no. 3: 464–469. https://doi.org/10.1111/j.1755-0998.2012.03112.x.

Hayatsu, H., S. Pan, and T. Ukita. 1971. "Reaction of Sodium Hypochlorite With Nucleic Acids and Their Constituents." *Chemical & Pharmaceutical Bulletin* 19, no. 10: 2189–2192. https://doi.org/10.1248/cpb.19.2189.

Hoffmann, A., V. Fingerle, and M. Noll. 2020. "Analysis of Tick Surface Decontamination Methods." *Microorganisms* 8, no. 7: 987. https://doi.org/10.3390/microorganisms8070987.

Huszarik, M., N. Röder, L. Eberhardt, et al. 2023. "External DNA Contamination and Efficiency of Bleach Decontamination for Arthropod Diet Analysis." *Environmental DNA* 5, no. 3: 540–550. https://doi.org/10.1002/edn3.410.

Kumar, G., E. Farrell, A. M. Reaume, J. A. Eble, and M. R. Gaither. 2022. "One Size Does Not Fit all: Tuning eDNA Protocols for High- and Low-Turbidity Water Sampling." *Environmental DNA* 4, no. 1: 167–180. https://doi.org/10.1002/edn3.235.

Mauvisseau, Q., A. Coignet, C. Delaunay, F. Pinet, D. Bouchon, and C. Souty-Grosset. 2018. "Environmental DNA as an Efficient Tool for Detecting Invasive Crayfishes in Freshwater Ponds." *Hydrobiologia* 805, no. 1: 163–175. https://doi.org/10.1007/s10750-017-3288-y.

Mauvisseau, Q., E. Kalogianni, B. Zimmerman, M. Bulling, R. Brys, and M. Sweet. 2020. "eDNA-Based Monitoring: Advancement in Management and Conservation of Critically Endangered Killifish Species." *Environmental DNA* 2, no. 4: 601–613. https://doi.org/10.1002/edn3.92.

Miya, M. 2022. "Environmental DNA Metabarcoding: A Novel Method for Biodiversity Monitoring of Marine Fish Communities." *Annual Review of Marine Science* 14, no. 1: 161–185. https://doi.org/10.1146/annurev-marine-041421-082251.

Nilsson, M., H. de Maeyer, and M. Allen. 2022. "Evaluation of Different Cleaning Strategies for Removal of Contaminating DNA Molecules." *Genes* 13, no. 1: 162. https://doi.org/10.3390/genes13010162.

Ohnishi, S., M. Murata, and S. Kawanishi. 2002. "DNA Damage Induced by Hypochlorite and Hypobromite With Reference to Inflammation-Associated Carcinogenesis." *Cancer Letters* 178, no. 1: 37–42. https://doi.org/10.1016/S0304-3835(01)00812-6.

Osinnikova, D. N., E. B. Moroshkina, and E. S. Mokronosova. 2019. "Effect of Sodium Hypochlorite on Nucleic Acids of Different Primary and Secondary Structures." *Journal of Physics: Conference Series* 1400, no. 3: 033001. https://doi.org/10.1088/1742-6596/1400/3/033001.

Peixoto, S., C. Chaves, G. Velo-Antón, P. Beja, and B. Egeter. 2021. "Species Detection From Aquatic eDNA: Assessing the Importance of Capture Methods." *Environmental DNA* 3, no. 2: 435–448. https://doi.org/10.1002/edn3.130.

Piaggio, A. J., R. M. Engeman, M. W. Hopken, et al. 2014. "Detecting an Elusive Invasive Species: A Diagnostic PCR to Detect Burmese Python in Florida Waters and an Assessment of Persistence of Environmental DNA." *Molecular Ecology Resources* 14, no. 2: 374–380. https://doi.org/10.1111/1755-0998.12180.

Prince, A. M., and L. Andrus. 1992. "PCR: How to Kill Unwanted DNA." *BioTechniques* 12, no. 3: 358–360.

Riley, A. 2019. "Bleach-Based Decontamination Treatment and Cytosine Deamination in Contaminant DNA." Master's Thesis. University of Tennessee. https://trace.tennessee.edu/utk_gradthes/5499.

Saccò, M., M. T. Guzik, M. van der Heyde, et al. 2022. "eDNA in Subterranean Ecosystems: Applications, Technical Aspects, and Future Prospects." *Science of the Total Environment* 820: 153223. https://doi.org/10.1016/J.SCITOTENV.2022.153223.

Shokere, L. A., M. J. Holden, and G. Ronald Jenkins. 2009. "Comparison of Fluorometric and Spectrophotometric DNA Quantification for Real-Time Quantitative PCR of Degraded DNA." *Food Control* 20, no. 4: 391–401. https://doi.org/10.1016/j.foodcont.2008.07.009.

Skinner, M., M. Murdoch, T. Loeza-Quintana, S. Crookes, and R. Hanner. 2020. "A Mesocosm Comparison of Laboratory-Based and On-Site eDNA Solutions for Detection and Quantification of Striped Bass (*Morone saxatilis*) in Marine Ecosystems." *Environmental DNA* 2, no. 3: 298–308. https://doi.org/10.1002/edn3.61.

Spens, J., A. R. Evans, D. Halfmaerten, et al. 2017. "Comparison of Capture and Storage Methods for Aqueous Macrobial eDNA Using an Optimized Extraction Protocol: Advantage of Enclosed Filter." *Methods in Ecology and Evolution* 8, no. 5: 635–645. https://doi.org/10.1111/2041-210X.12683.

Stoufer, S., M. Demokritou, D. Buckley, P. Teska, and M. D. Moore. 2023. "Evaluation of the Ability of Commercial Disinfectants to Degrade Free Nucleic Acid Commonly Targeted Using Molecular Diagnostics." *Journal of Hospital Infection* 133: 28–37. https://doi.org/10.1016/j.jhin.2022.12.010.

Thomsen, P. F., and E. Willerslev. 2015. "Environmental DNA—An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity." *Biological Conservation* 183: 4–18. https://doi.org/10.1016/J.BIOCON.2014.11.019.

Welsh, S., T. Peakman, S. Sheard, and R. Almond. 2017. "Comparison of DNA Quantification Methodology Used in the DNA Extraction Protocol for the UK Biobank Cohort." *BMC Genomics* 18, no. 1: 26. https://doi.org/10.1186/s12864-016-3391-x.

Whiteman, M., A. Jenner, and B. Halliwell. 1997. "Hypochlorous Acid-Induced Base Modifications in Isolated Calf Thymus DNA." *Chemical Research in Toxicology* 10, no. 11: 1240–1246. https://doi.org/10.1021/tx970086i.

Winter, J., M. Ilbert, P. C. F. Graf, D. Özcelik, and U. Jakob. 2008. "Bleach Activates a RedoxRegulated Chaperone by Oxidative Protein Unfolding." *Cell* 135, no. 4: 691–701. https://doi.org/10.1016/J.CELL.2008.09.024.

Yates, M. C., D. M. Glaser, J. R. Post, M. E. Cristescu, D. J. Fraser, and A. M. Derry. 2021. "The Relationship Between eDNA Particle Concentration and Organism Abundance in Nature Is Strengthened by Allometric Scaling." *Molecular Ecology* 30, no. 13: 3068–3082. https://doi.org/10.1111/mec.15543.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.